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Use of a carrier for quantitation of a new dihydropyridine calcium antagonist (OPC-13340) in human plasma by highly sensitive gas chromatography with negative-ion chemical ionization mass spectrometry

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ABSTRACT

A sensitive gas chromatographic–mass spectrometric method for the quantitation of (±)-methyl 3-phenyl-2(*E*)-propenyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (OPC-13340, I), a new dihydropyridine calcium antagonist with a potent and long-acting antihypertensive and antianginal effect, was developed in order to elucidate its pharmacokinetics. Dihydropyridine calcium antagonists have been usually quantified by this technique in the negative-ion chemical ionization mode. However, direct application of this method to quantify trace amounts of I in biological fluids completely failed, owing to its adsorption on the column and oxidation of its dihydropyridine ring. Human plasma containing I and (±)-[$^2H_3$]methyl 3-phenyl-2(*E*)-propenyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (II), the internal standard, was extracted with *n*-hexane–diethyl ether under weakly basic conditions (pH 8). In order to prevent adsorption of the compounds on the column, (±)-[$^2H_5$]ethyl 3-phenyl-2(*E*)-propenyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (III), an analogue of I, was added to the extracts as a carrier. In addition, this carrier was also effective in preventing the oxidation of I. The quantitation limit of I in human plasma by this method was found to be less than 30 pg/ml. Thus, the method is sufficiently sensitive to study the pharmacokinetics of I in humans.

INTRODUCTION

(±)-Methyl 3-phenyl-2(*E*)-propenyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (OPC-13340, I) is a newly developed dihydropyridine calcium antagonist, which exhibits potent and long-lasting antihypertensive and antianginal activities [1]. The antihypertensive activity of I is more potent than that of nicardipin, and is long-lasting enough to be used once daily in hypertension therapy.

In order to investigate the pharmacokinetics of I, a highly sensitive and specific method was required. A detection limit of less than 100 pg/ml was considered to be necessary because plasma concentrations of I were expected to be at the subnanogram level, owing to its low clinical doses of 1–4 mg per person per day. Consequently, conventional methods, such as high-performance liquid chromatography, were judged to be insufficiently sensitive and/or selective.

Recently, gas chromatography with electron-capture detection (GC–ECD) [2–9] and with negative-ion chemical ionization mass spectrometry (GC–NICI-MS) [10–12] have been widely used as sensitive and selective methods for quantitation of dihydropyridine calcium antagonists and their metabolites in biological fluids, because the nitro substituent on the phenyl group in most dihydropyridine calcium antagonists has a high electron

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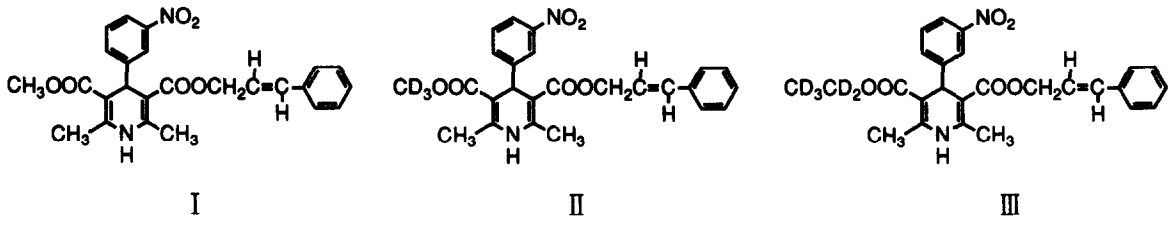


I II III

Fig. 1. Structures of OPC-13340 (I), internal standard (II) and carrier compound (III).

affinity and is suitable for the NICI mode to be used.

However, these methods [2,5,9] have the disadvantage that the analytes must be derivatized to more stable pyridine analogues because the dihydropyridine moiety decomposes during GC. Thus, the selectivity of these methods is spoiled because these pyridine analogues are already present in plasma as metabolites [8,9].

We tried to develop a highly sensitive and selective method for the quantition of I in human plasma. However, when picogram amounts of I were injected into a GC column, there was no peak corresponding to I in the chromatogram because of its adsorption on the column and oxidation. Hence an attempt was made to use III, which is an analogue of I, as a carrier [13,14]. This paper describes a simple, rapid and sensitive method for the quantitation of I in human plasma by GC–NICI-MS using III as a carrier to prevent adsorption and decomposition of I during GC analysis.

EXPERIMENTAL

Materials

Compounds I, II and III were synthesized at our institute (Fig. 1). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The other reagents and solvents were of analytical-reagent grade.

Gas chromatography–mass fragmentography

The GC–MS system consisted of a JEOL JMS SX102 coupled to a Hewlett-Packard Model 5890 gas chromatograph. A fused-silica capillary column coated with 5% phenylmethyl silicone (15 m × 0.32 mm I.D., film thickness 0.25 μm) was used. The column was directly connected to the ion source of the mass spectrometer, so that the end of the column was within 1.5 cm of the ion source centre. Helium was used as a carrier gas at an inlet pressure of 102 kPa for GC operation. The column temperature was maintained at 220°C for 0.6 min after a sample was injected. The split valve of split–splitless injector was closed before injection and opened for 0.6 min after injection. The column temperature was programmed to 300°C at 15°C/min, and maintained at that temperature for 4 min. The injector, the interface oven and the transfer line were operated at 290°C.

A mass spectrometer equipped with a pulsed NICI accessory was operated, using methane as a reagent gas at an ion source pressure of 0.01 Pa. The ionization current, ion source temperature, accelerating voltage and ionization energy were 0.3 mA, 250°C, −7 kV and 200 eV, respectively. A JMA-DA6000 data system was used for the selected-ion monitoring (SIM). The mass spectral resolution in the SIM mode was 500.

Sample preparation, standard preparation and analysis

Blood samples were collected in disposable culture tubes and immediately centrifuged for 15 min at 2000 *g* to separate plasma. To each 1.0 ml of plasma were added 10 μl of the internal standard solution (1 ng per 10 μl in acetonitrile) and 0.2 ml of 1 *M* sodium carbonate solution to adjust the pH *ca.* 8. The resulting solution was extracted with 5 ml of *n*-hexane–diethyl ether (1:1) for 15 min at room temperature. The extracts were dried under a stream of nitrogen gas. The residue was trimethylsilylated for 5 min at room

temperature by adding 20 μl of a solution of MSTFA in acetonitrile (3:100). The mixture was evaporated to dryness under a stream of nitrogen gas on a water-bath; the resulting residue was dissolved in 20 μl of an acetonitrile solution containing III (30 μg/ml), and the solution was used as a sample solution. Separately, acetonitrile solutions of I were prepared in concentrations of 3–1000 ng/ml, and 10 μl of these solutions were added to 1 ml of blank plasma. These standard plasma specimens were processed as described in the sample preparation, and the final solutions were used as standard solutions. A 1-μl volume of each of these solutions was then subjected to GC–NICI-MS analysis. A calibration curve was constructed with the standard solutions prior to the analysis of sample solutions.

RESULTS AND DISCUSSION

Mass spectra of I and its related analogues

Fig. 2 shows the NICI mass spectra of I and its $^2H_3$-labelled analogue using methane as a reagent gas. The ion at *m/z* 331 ($[M-CH_2CH=CHC_6H_5]^-$) appeared as the base peak, which accounted for 87% of the total ion intensity, whereas the molecular ion observed at *m/z* 447 was a minor peak. The base peaks at *m/z* 331 for I

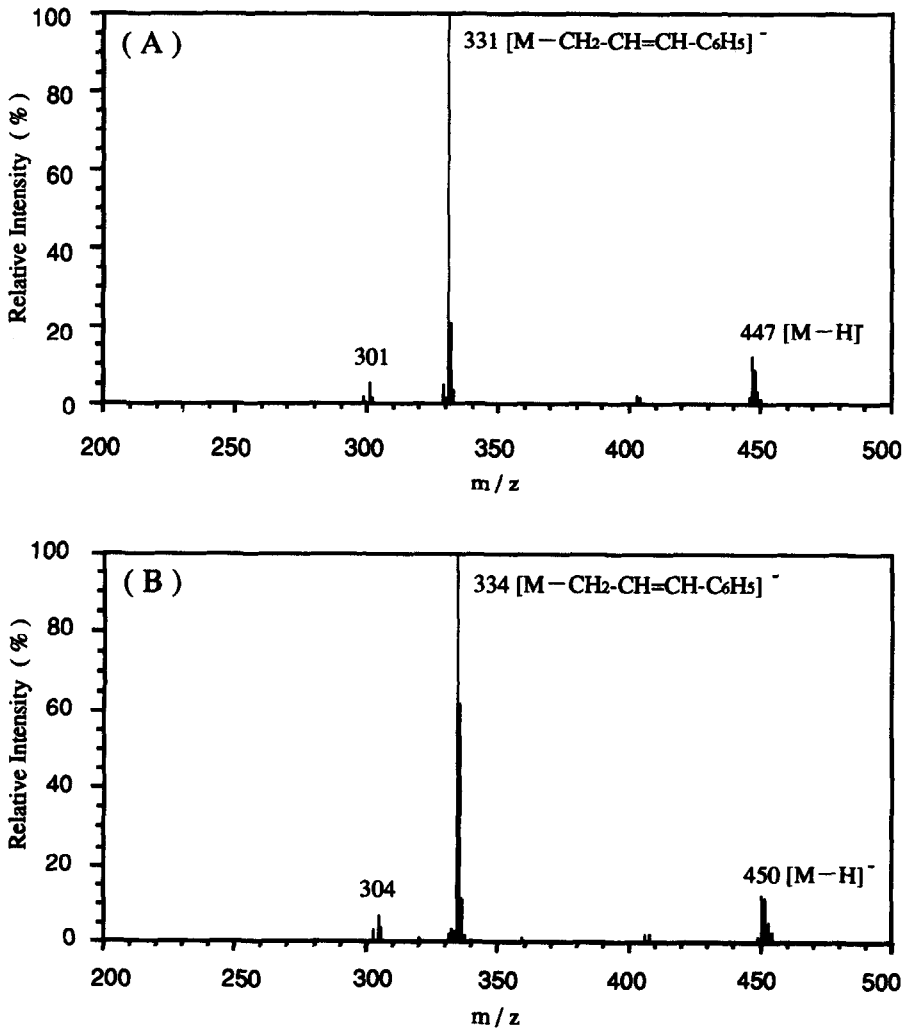
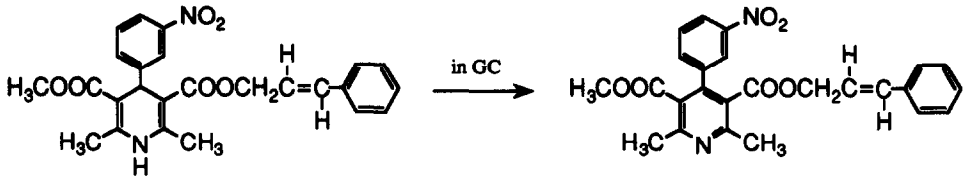


Fig. 2. NICI mass spectra of I (A) and II (B).



I Pyridine analogue

Fig. 3. Decomposition of I during GC.

and at *m/z* 334 for II were chosen as the monitoring ions for the quantitation of I by SIM.

Adsorption and oxidation of I during analysis

When low picogram levels of I were injected, there was no peak corresponding to I. This was thought to be due to adsorption of I on the column. In addition, we encounted the well known difficulty that dihydropyridine calcium antagonists decompose easily to pyridine analogues during GC–MS. In the cases of I, this undesirable oxidation (Fig. 3) was also observed in GC–NICI-MS: when 50 pg of I were subjected to GC–MS, the $[M-CH_2CH=CHC_6H_5]^-$ ion was observed at *m/z* 329 as a base peak, at 6 min after injection (Fig. 4). The retention time of the ion *m/z* 329 was identical with that of the authentic pyridine analogue, thus indicating that the oxidation of I occurs at the injection port.

Prevention of adsorption and oxidation

In order to develop a sensitive and selective method for I using GC–NICI-MS, it is essential to overcome the above-mentioned difficulties. For this purpose, the use of a carrier seemed to be promising [10,11]. Thus, III, a multi-deuterated analogue of the compound of interest, was used as a carrier.

Mixtures of 15 pg of I and various amounts (0–50 ng) of III were then analysed under the defined conditions, and the ions at *m/z* 331 for I with the retention time of 8 min and at *m/z* 329 for its pyridine analogue with the retention time of 6 min were monitored. As shown in Fig. 5, the peak area of *m/z* 331 for I increased from 12.0 without the carrier to 20.8 in the presence of 30 ng/μl of the carrier. On the other hand, the peak

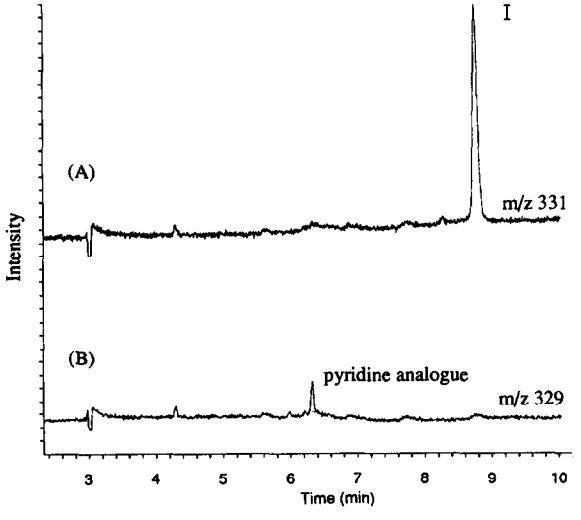


Fig. 4. GC–NICI selected-ion recordings after injection of 50 pg of I simultaneous monitoring at *m/z* 331 (A) for I and *m/z* 329 (B) for pyridine analogue.

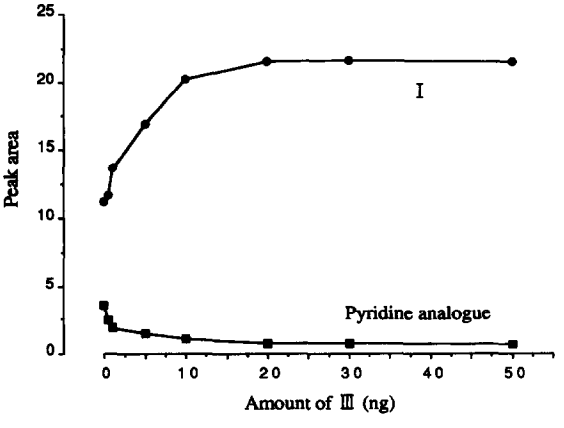


Fig. 5. Effect of III as a carrier added to the human plasma extract on prevention of adsorption and oxidation of I in GC–MS. Injection volume, 15 pg of I.

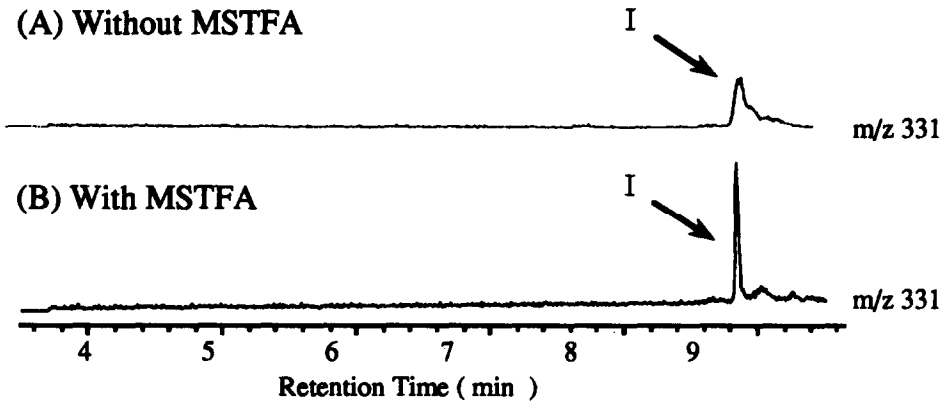


Fig. 6. Effect of MSTFA added to the extract from human plasma for improvement of the asymmetric peak in selected-ion chromatograms of I monitored at *m/z* 331.

area at *m/z* 329 for the pyridine analogue decreased from 3.2 without the carrier to 0.7 at 30 ng/μl of the carrier.

These results indicate that the use of a carrier is effective to prevent not only the adsorption of the analyte on the GC column, but also oxidation at the injection port of GC.

Refinement of the peak shapes

When the extract obtained from plasma samples spiked with I was subjected to GC–MS, an irregular and asymmetric peak appeared at *m/z* 331 (Fig. 6A). This was considered to be caused by adsorption of non-volatile impurities in the plasma extracts. To solve this problem, several silylating reagents were added to the extracts and injected into the system. MSTFA improved the asymmetric peak shape, as shown in Fig. 6B. However, the peak-area ratio of *m/z* 331 to *m/z* 334 was practically constant.

Calibration curve and recovery

A calibration curve was constructed using a series of mixtures of I (0.03, 0.1, 0.3, 1.0, 3.0, 10.0 ng), II (1.0 ng) and III (30 ng) in 1 ml of blank plasma.

The calibration curve gave a good linearity at concentrations from 0.03 to 10 ng/ml. The regression analysis revealed that the calibration curve was given by the equation $\log y = 0.9781 \log x + 0.2253$ (correlation coefficient = 0.998), where y is the ratio of the peak area of I to that of II and x represents the concentration in ng/ml. The recovery of I through the extraction procedure was found to be 88–94% at concentrations 0.03–10.0 ng/ml.

Table I shows the accuracy and precision of the present method using various concentrations of I in human plasma. The quantitation limits for I using the present method were 0.03 ng/ml in plasma.

TABLE I

ACCURACY AND PRECISION OF THE ASSAY OF I AT VARIOUS CONCENTRATIONS IN HUMAN PLASMA

Added concentration (ng/ml)	*n*	Found concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
0.03	5	0.034 ± 0.004	12.3
0.1	5	0.085 ± 0.011	12.9
0.3	5	0.304 ± 0.016	5.3
1.0	5	1.00 ± 0.052	5.2
3.0	4	3.05 ± 0.095	3.1
10.0	5	10.20 ± 0.38	3.7

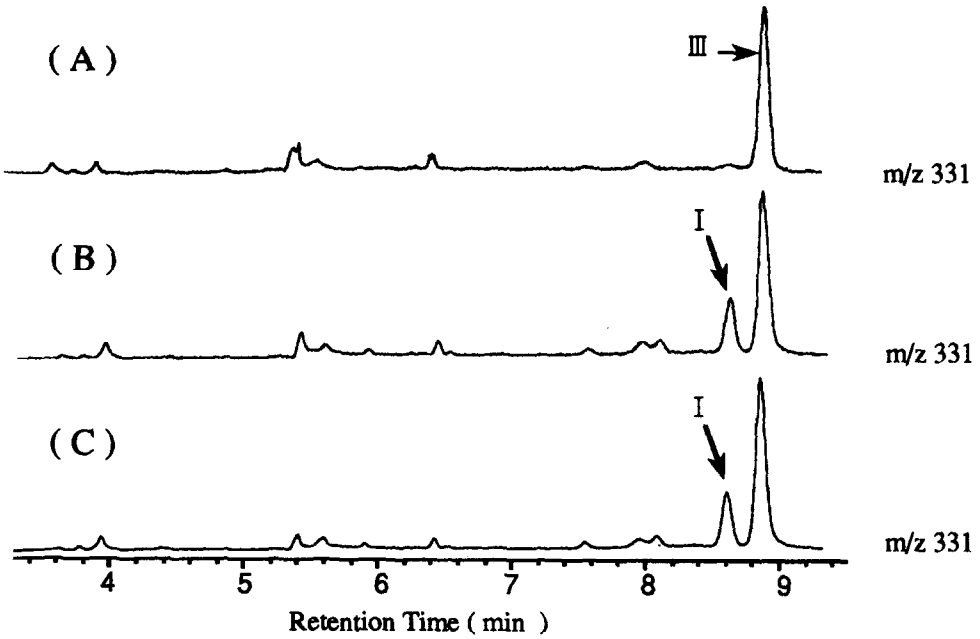


Fig. 7. Representative selected-ion recordings of extracts from (A) human blank plasma, (B) human plasma spiked with I (0.3 ng/ml) and (C) human plasma after an oral administration of 2 mg of I to a healthy volunteer.

Fig. 7A is the selected-ion recording of a blank plasma sample containing 1 ng/ml II and 30 ng/ml III. The peak of III corresponding to monitoring at *m/z* 331 was assigned to the ion with a very low intensity in this carrier. There were no interfering peaks in the blank plasma. Fig. 7B shows a selected-ion recording of a human plasma sample spiked with 0.3 ng/ml I. Fig. 7C shows

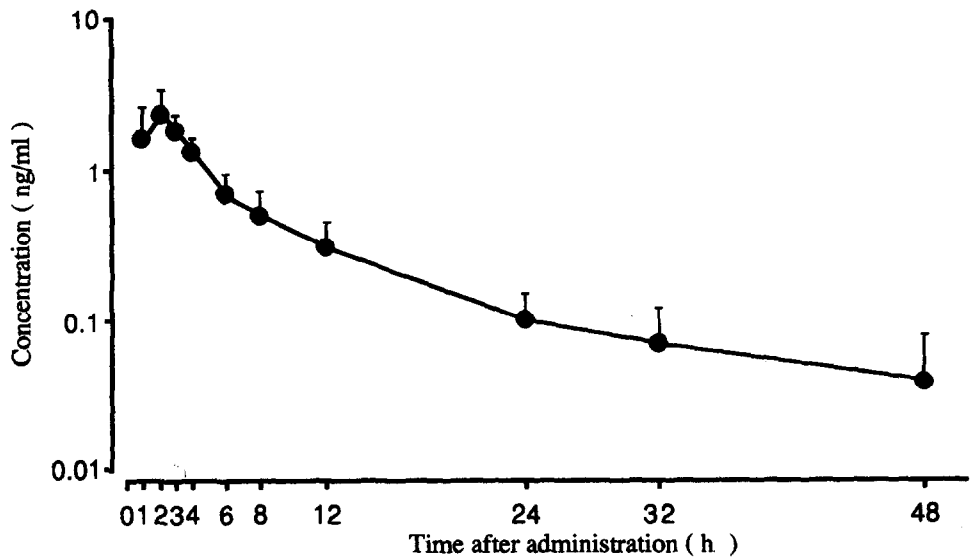


Fig. 8. Time course of I in plasma after oral administration of 2 mg to human subjects (each point represents the mean ± S.D. from twelve subjects).

a representative selected-ion recording of a human plasma sample obtained 12 h after oral administration of 2 mg of I to a healthy volunteer.

Fig. 8 shows an example of concentration–time profile of I in plasma after oral administration of 2 mg per person. The present method may be useful for the study of the pharmacokinetics of I in biological specimens.

In conclusion, the use of III as a carrier, which is an analogue of I, reduced adsorption and oxidation, and made it possible to quantify trace amounts of I in human plasma by SIM. The quantitation limit of I using the present method was found to be 30 pg/ml in human plasma.

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